MICROBIAL ACTIVITY IN GROUND WATER AT A CHEMICAL WASTE DISPOSAL SITE

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ABSTRACT: Selected ground water microbial processes were monitored in wells upslope and downslope from a research chemical waste disposal site on the campus of the University of Georgia. Burial of waste at the site was discontinued by the middle of the 1970's. Recently, it was determined that ground water at the site contained elevated levels of potentially toxic pollutants such as iron, manganese, benzene, methylene chloride, toluene, and xylene. Indices of microbial activity (e.g., uptake and mineralization of D-glucose) showed activity to be inhibited 2 to 5 fold in contaminated ground water relative to upslope control. Population switching experiments revealed no enhanced tolerance to the pollutant mixture of bacteria in the contaminated wells relative to control populations. However, rates of degradation of toluene, a compound of the pollutant mixture, were >20 fold higher in contaminated ground water than in control ground water, suggesting adaptation for enhanced degradation for a specific pollutant. Kinetics of toluene degradation by the microbial populations in the contaminated wells were multiphasic with the kinetic parameters K and V_{max} varying widely with substrate concentration. Such kinetic diversity has not been demonstrated previously for pollutant degradation in subsurface waters and has significant implications for modeling pollutant persistence and exposure.

INTRODUCTION

Ground water supplies the source of drinking water for more than 50% of the total U.S. population and nourishes many natural ecosystems valued for providing food resources, wildlife habitat, and recreation opportunities (Groundwater Protection, 1987). Most ground water supplies in the U.S. are of good quality. However, frequent discoveries of ground water contamination resulting from use, transportation, storage, and disposal of hazardous chemicals underline the need for studies of the fate and effects of these chemical pollutants in the subsurface.

Among the principal transforming processes, microbial degradation may be the only force which completely removes chemical pollutants from subsurface. Among environmental factors, inorganic nutrients and potential electron acceptors possess the potential to be manipulated to formulate bioremediation techniques. Lewis *et al.* (1986) and Swindoll *et al.* (1988) reported that adaptation lag periods prior to microbial transformation of organic chemicals at low concentrations correlated with limiting nutrient (N or P) concentrations for samples from surface and subsurface water, respectively. Transformations of certain halogenated compounds are likely to occur in anoxic environments in the presence of suitable electron acceptors (Bouwer and McCarty, 1983).

This study was designed to increase our understanding of the microbiological fate and effects of toxic organic chemicals in leachates derived from a waste landfill site near the University of Georgia. Those leachates contained high levels of dissolved hazardous chemicals such as toluene, xylene, benzene, and methylene chloride. To study the fate of these hazardous chemicals in such systems, the use of simplified laboratory studies of the biodegradation of individual compounds is often inappropriate in that complex interactions between and among the various chemicals can result in either enhancement or inhibition of the biodegradation of particular compounds. Moreover, the kinetics of the persistence of a compound based on degradation studies conducted over a narrow concentration range can be misleading and result in large errors if extrapolated to higher or lower concentrations (Hwang *et al.*, 1989). In this study we used two toxic compounds, *p*-cresol and toluene, as model pollutants and investigated the kinetics of their microbiological dissimilation over a wide range of substrate concentrations. In addition, the effects of inorganic nutrients (P and N), other chemical pollutants, and alternative electron acceptors (sulfate and nitrate) on microbial degradative activity in the ground water of the landfill site were assessed.

METHODS

Description of Study Site

The study site was the landfill located near the State of Georgia Botanical Garden at the University of Georgia. Burial at the landfill was initiated by about 1969 and was undertaken to meet the needs of numerous researchers to dispose of radioactive and chemical wastes. In the fall of 1972, the site was inspected and approved as a chemical burial site by the Georgia Environmental Protection Division. Thus, it served as the major repository of radioactive and chemical wastes generated at the University of Georgia. According to the chemical analyses conducted in November 1986, the dominant chemical species and their maximum concentrations in the ground water of contaminated sites were as follows: manganese (43 mg/L), iron (200 mg/L), naphthalene (150 µg/L), toluene (6.9 mg/L), trichloroethylene (490 µg/L), xylene (2.3 mg/L), methylene chloride (28 mg/L), 1,1,2,2-tetrachloroethane

(1.7 mg/L), formaldehyde (41 mg/L), benzene (15 mg/L), chloroform (40 mg/L).

Sample Collection and Analyses

Samples of ground water were obtained from a control well (upslope) and experimental wells (downslope) from the landfill, with aseptic teflon bailers (3 ft. long). The *in situ* oxygen concentrations in ground water samples were determined and care was taken not to introduce oxygen while transferring water from the bailers into acid-washed, argon-filled 10-liter polyethylene carboys. *In situ* dissolved oxygen concentration ranged from 11.2-11.5 mg/L for control water samples and 2.8-3.5 mg/L for experimental groundwater samples. pH ranged from 5.2-6.2 and 6.1-6.5 for control well samples and contaminated well samples, respectively. Temperature of ground water samples ranged from 10.5-11.0°C. Assays were initiated within 1 h of collection of the water samples.

The chemicals used in this study were (U-^{14}C)-labeled *p*-cresol, toluene, and D-[U-^{14}C]glucose.

The basic procedures for measurement of degradation kinetics, microbial biomass and activity were the same as those described previously (Hwang *et al.*, 1989).

The following analyses were conducted to determine the effect of ground water from the impacted site on microbial degradation of *p*-cresol. Bacteria from the ground water of control site were collected on 0.22-µm-pored Nuclepore filter and resuspended in filter-sterilized control-well water or impacted-well water. UL-^{14}C-*p*-cresol was added to the water samples at a final concentration of 10 µg/L and incubated at 24±1°C for 24 h. Microbial mineralization rates were measured and expressed as µg/L h^{-1}.

In separate experiments, varying amounts of chemical pollutants such as methylene chloride and toluene, of inorganic nutrients N & P (NH_4NO_3 and K_2HPO_4), and of electron acceptors such as sulfate and nitrate were added to the ground water samples from either the control well or the impacted well to assess the effects of candidate chemical pollutants, inorganic nutrients, and alternative electron acceptors on microbial degradation of *p*-cresol (10 µg/L).

RESULTS AND DISCUSSION

Effects on Microbial Degradation of *p*-cresol and Heterotrophic Activity

The effects of ground water from the impacted site on microbial degradation of organic chemical pollutants were assessed using *p*-cresol as the model compound. Indigenous microbial degradation rates of *p*-cresol in control-well and impacted-well water were 0.1 and 0.01 µg/L h^{-1}, respectively. However, microbial degradation rates decreased significantly if the microbial populations from the control well were resuspended in water from the impacted well. Assuming microbial degradative activities were additive, the observed integrated degradation rate (i.e., 0.03 µg/Lh^{-1}) by mixed microbial populations of the control and impacted sites indicated that microbial degradative activity of the control site was inhibited by 73% by exposure to ground water of the impacted site. Moreover, microbial utilization of glucose was 2 to 5 fold slower in the impacted well than that in the control well (Figure 1).

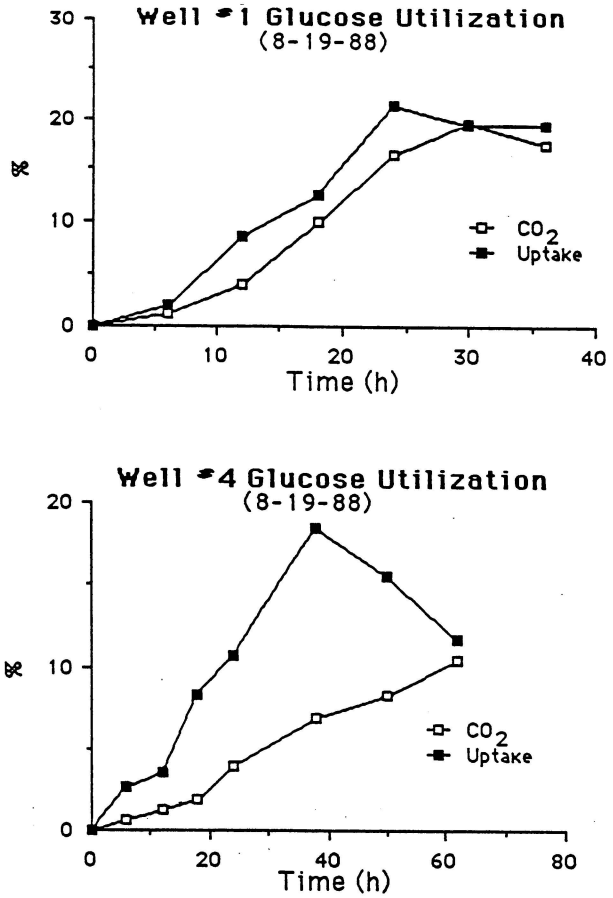

Figure 1. Microbial Utilization of Glucose.

Effect of Chemical Pollutants on Microbial Degradation of *p*-cresol and Heterotrophic Activities

Data from preliminary experiments revealed that seven of the seventeen chemical species in the contaminated ground water exhibited significant effects on microbial mineralization of *p*-cresol. Therefore, these seven chemicals were chosen for further studies. At the concentration reported for the impacted well, each of the chemical species inhibited microbial degradation of *p*-cresol, from 20% to 100%. Among the candidate chemicals, toluene, xylene, methylene chloride, and tetrachloroethane were the most inhibitory, inhibiting microbial degradation of *p*-cresol by virtually 100%.

The magnitude of inhibition by a given chemical varied with sampling date probably reflecting temporal changes in microbial populations in the subsurface. For example, microbial degradation of *p*-cresol in the unimpacted well was inhibited by Fe^{+3} by 43% in May. The magnitude of the inhibition, however, drastically increased to 93% in November (data not shown). Likewise, rates of microbial heterotrophic uptake of glucose and bacterial numbers (not shown) generally decreased in the presence of toluene, xylene, and tetrachloroethane, indicating that inhibition by these three compounds was due to general toxicity to the bacterial populations. Nevertheless, for the other chemical species the magnitude of the inhibition of heterotrophic activity did not correlate with that of inhibition of *p*-cresol degradation. Interestingly, *p*-cresol degradation at the

unimpacted site was inhibited by exposure to manganese and trichloroethylene, while the degradation rates at the impacted site were actually enhanced by 5-64%. Since general microbial activity parameters such as the total bacterial numbers and heterotrophic uptake of glucose did not increase with the exposure, the enhancement in *p*-cresol degradation can be assumed to be the result of a specific enhancement in degradation, possibly the induction of degradative enzyme(s).

Effect of Inorganic Nutrients and Alternative Electron Acceptors on Microbial Degradation of *p*-cresol

Among numerous environmental factors, inorganic nutrients and alternative electron acceptors were recently reported to have influence on microbial degradations of organic pollutants (Lewis *et al.*, 1986; Swindoll *et al.*, 1988; Bouwer and McCarty, 1983). N and P (0.14 mg/L of P as PO_4^{-3} and 11.5 mg/L of N as NO_3^-) were added to ground water of the impacted site to assess possible effects. However, there was no significant effect even if the concentrations of N and P were doubled (data not shown). Therefore, we may assume that microbial degradative activities at the landfill were not limited by the depletion of inorganic nutrients N and P.

For assessing the effects of alternative electron acceptors on anaerobic microbial degradation of chemical pollutants, both sulfate and nitrate were added owing to the reported existence of anaerobic metabolism of aromatic compounds by sulfate reducers and/or denitrifiers in subsurface waters (Bouwer and McCarty 1983; Evans and Fuchs, 1988). However, the results of sulfate addition on *p*-cresol degradation were inconsistent. When sodium sulfate was added at concentrations ranging from 1 mg/L to 100 mg/L in August, microbial mineralization of *p*-cresol was enhanced by 100-300%. Furthermore, addition of molybdate [i.e., an inhibitor of sulfate reducers (Oremland and Capone, 1988)] at concentrations ranging from 1-100 mg/L decreased microbial mineralization of *p*-cresol 18-64%. This inhibition, however, was reversible by the addition of sulfate. The results of this experiment, therefore, suggested that the sulfate reducers contributed to the total microbial degradation of *p*-cresol. However, the results were not repeatable when a similar experiment was conducted in September. The addition of sodium sulfate at a concentration of 100 mg/L inhibited *p*-cresol degradation by 25%, and the addition of molybdate (200 mg/L) or the addition of a combination of sulfate and molybdate did not significantly affect the degradation rate (data not shown). One possible explanation is that dominant microbial populations shifted temporally between sulfate reducing bacteria and bacteria of other metabolic type(s), with sulfate reducers being dominant in August and other metabolic type(s) in September.

When ammonium nitrate was added at concentrations between 10 and 100 mg/L, *p*-cresol mineralization was enhanced by 20% to 50% while the total bacterial numbers remained constant throughout incubation. Enhancement of the degradation rate was more significant (up to 230%), with the addition of nitrate in the form of potassium nitrate in the same month (i.e., September). The total bacterial numbers, however, sometimes increased with the addition of potassium nitrate. Nitrate respiration and concommitant transformation of toxic organic chemicals has been reported under conditions favoring denitrification where the redox

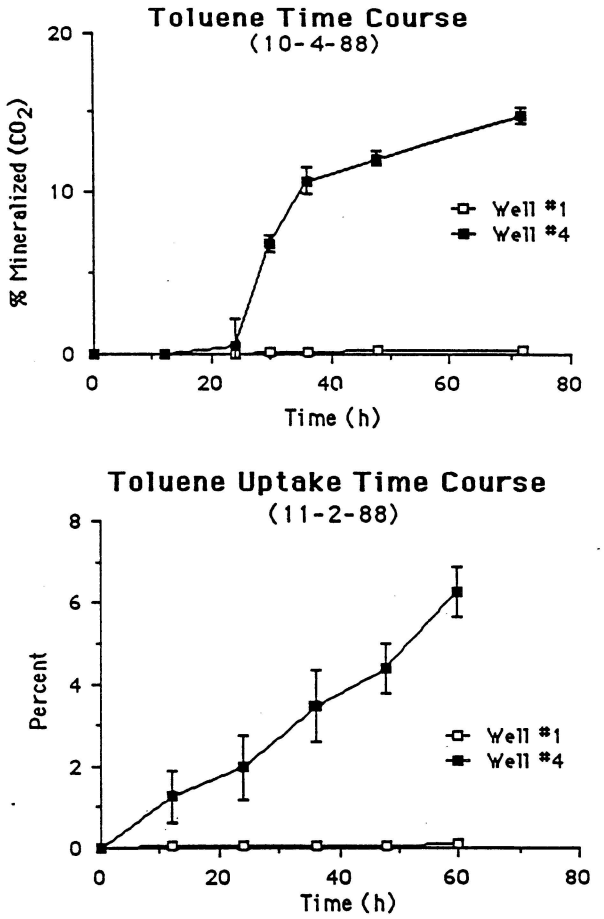

Figure 2. Microbial Degradation of Toluene.

condition was between that for aerobic and methanogenic transformation (Evans, 1977; Bouwer and McCarty, 1983). Although ground water of the impacted site was not anaerobic (concentration of the dissolved oxygen was about 3 mg/L), denitrification could occur in micro-anaerobic zones. We assume that potassium nitrate might serve both as a nutrient and an electron acceptor for the microbial community of the impacted site.

Microbial Degradation Kinetics of Toluene in the Ground Water

Microbial degradation of toluene was negligible in the control well, while rates of degradation were >20 fold higher in the contaminated well (Figure 2), suggesting adaptation for enhanced degradation for specific pollutants. Our degradation kinetic data have been linearlized according to the method of Wright and Hobbie (1965, 1966), when concentration of added substrate [A] is plotted against t/f (incubation time, t, divided by fraction of substrate utilized, f). In this representation, if the data fall along a straight line, degradation is assumed to be mediated by a single saturable system with a single K_t (intercept of line on the x-axis) and V_{max} (inverse of the slope). If the data fall along a curved line with slope decreasing as [A] increases, we may assume that the microbial population is kinetically diverse with multiple uptake/degradative systems (see Hwang *et al.*,

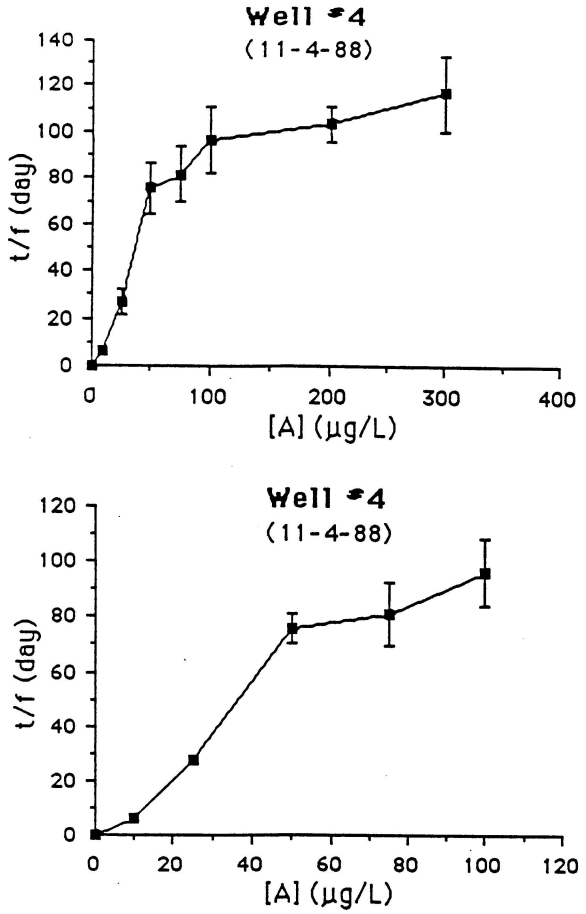

Figure 3. Wright-Hobbie Plot of Toluene Mineralization.

1989). Such multiphasic kinetics were seen repeatedly for toluene degradation in water from the impacted well (Figure 3). Such kinetic diversity has not been reported for ground water microbial populations.

SUMMARY

General microbial activity was 10-fold lower in the impacted well than that in the control well. However, even in the impacted well, multiphasic kinetic patterns of pollutant degradation were observed, owing to the heterogeneity of degradative systems of the indigenous microbial assemblages. The mixture of candidate chemical species exhibited toxicity to bacterial general metabolic functions at the *in situ* concentrations. However, enhancement of bacterial degradation of toluene was observed, due to adaptation by exposure to the specific chemical pollutant in the leachates from the landfill. Moreover, alternative electron acceptors such as sulfate and nitrate increased microbial transformation of *p*-cresol under *in situ* redox conditions. Therefore, addition of an alternative electron acceptor or a combination of them may have the potential for *in situ* bioremediation of contaminated ground water at the landfill.

ACKNOWLEGMENTS

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LITERATURE CITED

Bouwer, E. J., and P. L. Mc Carty. 1983. Transformations ofhalogenatedorganic compounds under denitrification conditions. Applied and Environmental Microbiology 45: 1295-1299.

Evans, W. C. 1977. Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. Nature (London) 270: 17-22.

Evans, W. C., and G. Fuchs. 1988. Anaerobic degradation of aromatic compounds. vol. 42, *In* : Annual Review of Microbiology. L. N. Ornston, A. Balows and P. Baumann (Editors). Annual Reviews, Inc., Palo Alto, California. pp. 289-317.

Hwang, H.-M., R. E. Hodson, and D. L. Lewis. 1989. Microbial degradation kinetics of toxic organic chemicals over a wide range of concentartions in natural aquatic systems. Environmental Toxicology and Chemistry 8: 65-74.

Lewis, D. L., H. P. Kollig, and R. E. Hodson. 1986. Nutrient limitations and adaptation of microbial populations to chemical transformations. Applied and Environmental Microbiology 51: 598-603.

Oremland, R. S., and D. G. Capone. 1988. Use of "specific" inhibitors in biogeochemistry and microbial ecology. vol. 10, *In* : Advances in Microbial Ecology, K. C. Marshall (Editor). Plenum Press, New York, New York, pp. 285-383.

Swindoll, C. M., C. M. Aelion, and F. K. Pfaender. 1988. Influence of inorganic and organic nutrients on aerobic biodegradation and on the adaptation response of subsurface microbial communities. Applied and Environmental Microbiology 54: 212-217.

The Conservation Foundation (Editor). 1987. Groundwater Protection. The Conservation Foundation, Washington, D.C.

Wright, R. T., and J. E. Hobbie. 1965. The uptake of organic solutes in lake water. Limnology and Oceanography 10: 22-28.

Wright, R. T., and J. E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosysytems. Eology. 47: 447-464.